REMARKS/ARGUMENTS

Status of the Application

In the October 10, 2006, Non-Final Office Action (*hereinafter* "Office Action"), claims 13-20 were rejected. In the present response, claims 13, 14, 19, and 20 were amended to correct grammatical errors, for clarity, and to remove redundancies. No new matter was added.

Rejections Under 35 U.S.C. § 112, 2nd Paragraph

Claims 13-20 were rejected under 35 U.S.C. § 112, 2nd Paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Applicants address these rejections below.

Claim 13 and its dependent claims were rejected for "the recitation of 'regions of homology to different portions of a P1 donor cell chromosome' since it is not clear what is intended by 'regions of homology', and furthermore, what is being referred to by the term 'different portions'." Office Action at page 2, ¶ 5. Claim 13 was also rejected for the

> recitation of 'at least one donor cell' in part b), and 'infecting the transformed donor cell of (b)' in part c), and 'a set for donor cells of (c)' in e);infecting [sic] a recipient cell' in part f), 'selecting transduced recipient cells' in part g), 'screening the recipient cell of (f)' in part h), etc., since it is not clear how a single cell can be subjected to the recited steps, and furthermore, the steps seem to switch between the singular 'a cell' and the plural 'cells', which renders the claim unclear.

Id. at page 3, carryover paragraph. Claim 13 was further rejected because it is allegedly unclear in step h) "whether the selection step of g) has been performed on the recipient cells that are screened . . . ." *Id.*

Regarding the term "regions of homology", Applicants respectfully submit that the specification makes it clear to one skilled in the art what is meant by the term. The term "homology arm" is defined at page 9, lines 1-5 as "a nucleotide sequence that enables homologous recombination between two nucleic acids having substantially the same nucleotide sequence in a particular region of two different nucleic acids." Preferred size ranges and percent identities follow. "Homology" is defined as "nucleotide sequences sharing identical or nearly identical sequences" (page 10, lines 4-6). Typical integration cassette structures are set forth at page 17, lines 19-26 and page 17, line 35 – page 18, line 2. Example 1 and Figure 3

exemplify homologous recombination via λRed recombinase using a 40-50 basepair region of homology, and the use of λRed recombinase is further explained at page 7, lines 16-18 and page 20, line 31 – page 21, line 13. Thus, a region of homology as used in the claim is determined based on what host and where in the genome of said host the practitioner of the present invention wants a homologous recombination event to occur. The practitioner will know, with some certainty (at least 70% sequence identity, see page 9, lines 7-11), the target sequence in the host organism and will design the region of homology in an integration cassette accordingly.

The term "different portions" must be read in context with the remainder of step (a), namely that *each cassette comprises regions of homology to different portions of a P1 donor cell chromosome*. The key to interpreting this step is thus that each cassette must have a different region of homology, which is explained in detail at page 7, line 23 – page 8, line 6. Essentially, if there is only one region of homology, then only one genetic trait can be moved to a recipient cell (i.e., conventional P1 transduction). By having different regions of homology, however, Applicants' claimed invention allows for movement of multiple traits from a P1 donor cell in parallel combinatorial fashion to a recipient cell. Figure 2 illustrates this effect quite clearly. As seen under the heading "Combinatorial P1 transduction", four different integration cassettes have been inserted into the genome of the donor cells. One round of transduction and homologous recombination in the recipient cell produces chromosomal integrations having multiple traits, and the recipient cell can then be further screened and reused as a recipient cell to add further traits through additional round(s) of transduction and homologous recombination. In contrast, conventional P1 transduction as illustrated in Figure 2 will result in the movement of only one genetic trait at a time into a recipient cell.

Regarding the Examiner's concerns about steps b) – h) of claim 13 and the use of singular and plural terms, Applicants respectfully submit that the present amendments to the claim clarify the scope of the claimed method (support for the amendments can be found throughout the specification). The amended claim clearly describes the claimed method, summarized as follows. A multiplicity of integration cassettes are provided, at least one of which is chromosomally integrated into at least one donor cell. At least one of these transformed donor cells is infected with a P1 phage, at least one of these transformed and infected cells is lysed, and the

phage isolated from the lysate. Isolated phage from a set of donor cells, i.e., donor cells having different integration cassettes integrated into their chromosomes, are mixed, with the mixture being used to infect at least one recipient cell. The at least one recipient cell is grown so that a population of recipient cells having the selectable marker is produced, and these recipient cells are selected for screening based on their production of the selectable marker. A first overproducing strain is the recipient cell having the selectable marker that produces the highest level of genetic end product. The selectable marker is excised from the chromosomally integrated cassette in the first overproducing strain, which in turn is infected with isolated phage from the set of donor cells mentioned above. The first overproducing strain is grown so that a population of first overproducing strain having the selected marker is produced, and members of the population having the selectable marker are selected for screening. A second overproducing strain is the member of the population of first overproducing strain having the selectable marker producing the highest level of genetic end product. The first and second overproducing strains are then compared to optimize production of the genetic end product.

In light of the amendments to claim 13 and the above explanation, Applicants respectfully submit that the steps of claim 13 are clear and definite to one skilled in the art.

Applicants also respectfully submit that the present amendments to claim 13 obviate the rejection based on language used in step h) (now step i)). Step i) has been amended to state that the transduced recipient cell of step h) instead of step f) is screened for the highest level of the genetic end product to identify a first overproducing strain. This amendment should alleviate the Examiner's concern about clarity in relation to this step of claim 13.

Claim 14 was rejected for recitation of the term "derived from a cell" because of an alleged lack of a definition for the word "derived". Office Action at page 3, ¶ 1. Claim 14 was also rejected for "the recitation of 'wherein the promoter regions are . . .' since the claim on which the claim depends, i.e. claim 13, recites 'promoter' in the singular form; therefore it cannot be determined what is intended by the claim." *Id.* at page 3, ¶ 2. Applicants have amended claim 14 to recite that "each promoter is a native promoter of a cell other than the donor cell or recipient cell." Through this amendment, Applicants have clarified that each promoter is native to a cell other

than the donor or recipient cells (see, e.g., page 11, line 26 – page 12, line 5; page 17, lines 30-34; page 18, line 9-13 for support). Further, utilization of the term "each promoter" in claim 14 should remove doubt as to the number of promoters in claim because claim 13 step a) indicates that there are a multiplicity of integration cassettes used in the invention, with each integration cassette comprising a promoter. Therefore, there is a multiplicity (i.e., at least two) of promoters used in the claimed method, which is captured by the term "each promoter" in amended claim 14.

Claim 19 was rejected for "the recitation of 'the genes of the isoprenoid biosynthetic pathway are selected from the group consisting of . . .' since the claim on which this claim depends, i.e. claim 18, recites 'a gene', rather than the plural 'genes'." Office Action at page 4, ¶ 1. In response thereto, Claim 19 has been amended to recite the term "the gene of the isoprenoid biosynthetic pathway" as found in claim 18.

Summary

In view of the foregoing amendments and remarks, Applicants submit that this application is in condition for allowance. In order to expedite disposition of this case, the Examiner is invited to contact Applicants' representative at the telephone number below to resolve any remaining issues. Should there be a fee due which is not accounted for, please charge such fee to Deposit Account No. 04-1928 (E.I. du Pont de Nemours and Company).

Respectfully submitted,

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